Cis-Urocanic Acid, a Product Formed by Ultraviolet B Irradiation of the Skin, Initiates an Antigen Presentation Defect in Splenic Dendritic Cells In Vivo

Frances P. Noonan, Ph.D., Edward C. De Fabo, Ph.D., and Harry Morrison, Ph.D.

Clinical Microbiology, School of Medicine, Flinders University of South Australia, Adelaide, South Australia; Department of Dermatology, George Washington University, Washington, D.C., U.S.A.; Department of Chemistry, Purdue University, Lafayette, Indiana, U.S.A.

Urocanic acid (UCA, deaminated histidine) is a major ultraviolet-absorbing component of the stratum corneum. On UV irradiation, the naturally occurring trans form converts to the cis isomer. We have previously postulated that UV-induced systemic suppression is initiated by cis-UCA by way of an antigen-presenting cell defect. To test this hypothesis further, we have investigated the antigen-presenting cell (APC) function of splenic dendritic cells (DC). Splenic DC were prepared from mice 7 days after 1 h UV irradiation (27 kJ/m^2) or i.v. administration of 50–200 μg/mouse of cis- or trans-UCA. Dendritic cells from UV-irradiated or cis-UCA-treated mice had a significantly impaired (APC) ability, assessed by the proliferative response of purified T cells from mice immune to DNP_6 OVA to DC pulsed with this antigen. Dendritic cells from mice given trans-UCA had normal APC ability. The number of FcR+ cells was the same in DCs from all four treatment groups, and the number of IA^d+ cells and the intensity of IA^d expression were not decreased in DCs from UV-irradiated or cis-UCA-treated mice. Mixture of DCs from UV- or cis-UCA-treated mice with DCs from normal mice did not suppress APC activity. Dendritic cells taken 3 days after UV or cis-UCA treatment, in contrast to DC taken 7 days after treatment, had normal APC ability, indicating a time delay in the generation of the APC defect. In contrast, addition of cis-UCA or trans-UCA (66 μg/ml) directly to an in vitro proliferation assay had no effect, suggesting that cis-UCA may be activated in vivo. These results support our original hypothesis that cis-UCA has a natural role as a modulator of immune function. *J Invest Dermatol 90:92–99, 1988*

Ultraviolet B (UVB) irradiation of mice causes a systemic immune suppression that modulates contact hypersensitivity responses [1,2], delayed-type hypersensitivity responses to viruses [3] and to parasites [4], and that may be a critical step in UV carcinogenesis, preventing the immunologic rejection of highly antigenic UV tumors [5,6]. Recent studies have indicated that UVB irradiation can also modulate the course of certain autoimmune diseases [7,8]. The mechanism by which UVB causes immune suppression has not yet been established.

We have previously reported evidence from a wavelength dependence study of UV-induced suppression of contact hypersensitivity that suppression is initiated by an interaction between UVB radiation and a specific signal-transducing photoreceptor molecule in mouse skin [9]. We put forward the hypothesis that the photoreceptor molecule is the trans isomer of urocanic acid (UCA, deaminated histidine), a major UV-absorbing component of the stratum corneum [9]. In this report, we have tested this hypothesis further by investigating the premise that the UV-induced cis isomer of UCA interacts with the immune system.

UCA or deaminated histidine (4-imidazole acrylic acid) is a product formed in the stratum corneum by the action of the enzyme histidase [10]. UCA is naturally present in the skin as the trans isomer, but, on UVB irradiation in vitro [11] or in the skin [12], the cis form is produced. The prediction from our hypothesis is that the cis isomer of UCA interacts with the immune system, initiating immunosuppression.

UVB irradiation of mice in vivo has two well-described effects on the immune system. The splenic antigen presenting cells are markedly functionally deficient [13,14], and, when antigen is given to an UV-irradiated mouse, antigen-specific suppressor T cells are formed [1,5,6,13]. It has been hypothesized that these two events are causally related [13,14]. We have previously proposed a scheme in which the antigen-presenting cell defect and the resultant formation of antigen-specific suppressor T cells are initiated by the UV-induced formation of the cis isomer of UCA [9,34].

In this paper, we show that administration of cis-UCA to mice induces one of the effects of in vivo UV irradiation, the formation of

Manuscript received October 27, 1986; accepted for publication July 3, 1987.

Reprint requests to: Dr. F. Noonan, Department of Dermatology, George Washington University Medical Center, Ross Hall, 2300 Eye St., NW, Washington, DC 20037, USA.

This work was done while Frances P. Noonan was the B.S. Hanson Research Fellow of the Anti-Cancer Foundation of the Universities of South Australia. Research was also supported by grants from the Anti-Cancer Foundation of the Universities of South Australia, and from National Health and Medical Research Council of Australia, and the Flinders Medical Center Research Foundation, and in part by PHS Grant No. CA 18267 awarded by NCI, DHHS.

Abbreviations:

APC: antigen presenting cell
BSA: bovine serum albumen, Fraction V
DC: dendritic cell
DNP_6 OVA: ovalbumin conjugated with 6 dinitrophenyl groups
FACS: fluorescence activated cell sorter
FcR: receptor for Fc region of immunoglobulin
FITC: fluorescein isothiocyanate
HPLC: high-performance liquid chromatography
PBS: phosphate buffered saline iso-osmotic with mouse serum
UCA: urocanic acid or 4-imidazole acrylic acid
UVB: ultraviolet B radiation (290–320nm)

0020-202X/88/$03.50

functionally deficient splenic antigen presenting cells (APC). Purified splenic dendritic cells (DC) from UV-irradiated mice or from mice given cis-UCA have a decreased ability to present antigen to immune T cells in an in vitro proliferation assay. The antigen presentation defect was detectable in DC taken 7 days after either UV irradiation or cis-UCA administration. This finding is consistent with and lends support to our hypothesis that cis-UCA has a natural role as a modulator of immune function.

MATERIALS AND METHODS

Mice BALB/c mice were supplied by the Animal Resource Centre of the Department of Agriculture, South Australia, and were used according to the ethical guidelines of the National Health and Medical Research Council and the Commonwealth Scientific and Industrial Research Organisation of Australia. Mice were 8–12 wk old at the start of experiments.

UV Irradiation Mice had their backs shaved and were UV irradiated for 1 h as described previously [1]. The UV source was a bank of FS40 sunlamps emitting a broad band of UV radiation (250–360 nm) with 65% of the output in the UVB range (290–320 nm) [15]. The dose-rate monitored with a UVX Radiometer and a UVX-31 sensor (Ultraviolet Products Inc., San Gabriel, California) was 7.5 $J/m^2/s$; the total dose delivered in 1 h was 27 kJ/m^2.

Urocanic Acid Trans-UCA (4-imidazole acrylic acid) was purchased from Sigma Chemical Co., St Louis, Missouri. Cis-UCA was prepared by UV irradiation of trans-UCA and was purified by HPLC as described previously [16]. Purity of cis- and trans-UCA was confirmed by an alternative HPLC method [17]; no detectable cross-contamination of isomers was found.

Administration of UCA In Vivo Trans-UCA or cis-UCA was dissolved in sterile phosphate-buffered saline solution iso-osomotic with mouse serum (PBS) and injected intravenously, as indicated, in a 1-ml volume. Mice had their backs shaved prior to injection of UCA to mimic the status of UV irradiated mice. The in vivo doses of UCA used in this study (50–200 μg/mouse) were designed to approximate the amount of cis-UCA generated by an immunosuppressive dose of UV radiation in vivo as estimated by two methods: *(a)* the amount of cis-UCA theoretically generated by a dose of narrow-band UV (270 ± 1.5 nm) sufficient to give 70% systemic suppression of CHS (420 J/m^2) [9] is 80 μg/mouse, assuming a quantum yield of 0.5 and an in vivo photostationary state of trans to cis isomer of 0.5; *(b)* the amount of cis-UCA detected by HPLC [17] in an extract of mouse skin that had been irradiated in vivo with 1 h of UV radiation from FS40 sunlamps, a dose that causes 70%–80% suppression of CHS [1] is 120 μg/mouse (unpublished observations).

Preparation of Splenic Dendritic Cells Splenic dendritic cells (DCs) were prepared by the method of Steinman and Cohn [18]. Single cell suspensions of 5–10 mouse spleens were prepared and suspended in a solution of bovine albumen fraction V (Sigma Chemical Co., St Louis, Missouri), density 1.088, pH 7.35. The suspensions were overlayed with medium and centrifuged at 10,000g for 30 min. The cells at the interface were further enriched for DCs by adherence to plastic tissue culture dishes (Disposable Products, Adelaide, South Australia), at 37°C in an atmosphere of 5% CO_2 in air. After 1–2 h, the nonadherent cells were discarded. The cells that detached after a further overnight incubation at 37°C were harvested and used in most experiments. The medium used in all experiments was RPMI 1640 supplemented with 10% heat inactivated fetal calf serum (Flow Laboratories, North Ryde, New South Wales, Australia), 200 mM glutamine, 10 mM Hepes, 100 U/ml penicillin and 100 μg/ml streptomycin. The DCs prepared were 65%–90% IA positive, 8%–11% Fc receptor positive, <2% positive with anti-Thy 1.2, and <10% weakly positive with anti-Ig.

Immunization of Mice Mice were immunized at four sites (two footpads and two subcutaneous sites) with a total of 20 μg per mouse of DNP_6 OVA prepared from OVA (Calbiochem, La Jolla, California) according to the method of Eisen [19] and emulsified with complete Freund's adjuvant.

Purification of T Lymphocytes A three-step procedure was used. Popliteal, inguinal, and axillary lymph nodes from 3–6 immunized mice were taken, and single cell suspensions were prepared by using a cell sieve. The lymphoid cells were incubated on nylon wool columns for 1 h at 37°C [20], and the nonadherent cells eluted from the column adhered to plastic tissue culture dishes for 1 h at 37°C. The nonadherent cells from the tissue culture dish were incubated on a Sephadex G-10 column [21] for 1 h at 37°C. The eluted cells were >95% T cells, as tested by fluorescent staining using a monoclonal antibody to Thy 1.2., and gave minimal responses to antigen in the absence of added APC.

Proliferation Assay The method of Stingl et al [22] was followed with some modifications: 4×10^5 purified T cells from immunized mice were cultured alone, with antigen or with antigen-presenting cells. Dendritic cells were pulsed either with culture medium or with antigen (DNP_6 OVA, 100 μg/ml) at a concentration of 1×10^6 cells/ml at 37°C in 5% CO_2 in air. Cells were washed four times before addition to the proliferation assay. Triplicate cultures were maintained in 96-well flat-bottomed tissue culture dishes (Linbro, McLean, Virginia) for 3 days in fully supplemented RPMI medium with 5×10^{-5}M mercaptoethanol. At the end of the third day, 1 μCi/well of tritiated thymidine (5 Ci/mmol, Amersham, Buckinghamshire, United Kingdom) was added; the next morning cells were harvested in a multiple automated cell harvester (Skatron, Lieberbyen, Norway), and tritiated thymidine uptake was determined by using liquid scintillation counting. Statistical significance was determined using Student's *t* test.

Immunofluorescence Cells were labeled by a two-stage immunofluorescence procedure. For the first step, culture supernatants from the following mouse or rat hybridomas were used (American Type Culture Collection, Rockville, Maryland): Anti-IA^d (HB3; MK-D6;23) or anti-Thy 1.2 (TIB 107). Cells were visualized using as a second antibody either FITC labeled goat anti-mouse Ig or FITC rabbit anti-rat Ig (Nordic, Tilburg, Netherlands). Fluorescent cells were counted by flow cytometry using a fluorescence activated cell sorter (FACS IV, Becton Dickinson).

Rosetting The number of Fc receptor-positive cells was enumerated by rosetting with hemolysin (MA Bioproducts, Walkersville, Maryland) and sheep red blood cells [18]. The percentage of rosetting cells was determined by visual count using a light microscope.

RESULTS

Antigen Presentation by Dendritic Cells is Unaffected by the Addition of Urocanic Acid In Vitro As a first step in the investigation of a possible effect of UCA on antigen presentation, we tested the premise that UCA might interfere directly with the presentation of antigen to T cells. Cis-UCA or trans-UCA was added in vitro to a proliferation assay. In this assay, (see Materials and Methods), culture of DCs pulsed with medium and immune T cells resulted in a syngeneic proliferative response; co-culture of antigen pulsed DC and immune T cells gave an antigen-specific proliferative response three-to four-fold higher than the syngeneic response (Table I). Purified DCs cultured alone for 3 days gave a proliferative response of <700 cpm. Addition of either cis-UCA or trans-UCA (66 μg/ml) to the cultures at day 0 had no effect on either the syngeneic or the antigen-specific proliferative responses (Table I). Other studies (Noonan et al., unpublished) also revealed a similar lack of effect of cis- or trans-UCA on in vitro proliferation assays when either epidermal Langerhans cell containing preparations or plastic adherent peritoneal cells were used as APC.

Antigen Presenting Cell Function of Splenic Dendritic Cells after Ultraviolet B Irradiation of Mice Previous studies [13,14] had shown that splenic adherent cells, (a heteregeneous population containing macrophages, dendritic cells, and lympho-

Table I. Addition of UCA In Vitro Does Not Alter the Syngeneic or the Antigen-Specific Proliferative Response of T Cells to Dendritic Cells

	[^{3}H]Thymidine incorporated[b]			
	DCs pulsed with medium		DCs pulsed with DNP_6OVA[c]	
Cells cultured[a]	Expt 1	Expt 2	Expt 1	Expt 2
T cells + DC	11.1 (2.6)	17.6 (0.3)	40.3 (5.2)	42.6 (2.3)
T cells + DC + trans-UCA (66 μg/ml)	8.7 (0.7)	—	27.9 (4.3)	38.2 (2.4)
T cells + DC + cis UCA (66 μg/ml)	9.5 (1.3)	—	35.8 (6.6)	46.2 (5.6)

[a] 4×10^5 purified T cells from mice immunized with DNP_6OVA in CFA were cultured for 3 days alone or with 1×10^4 purified DCs from normal mice, pulsed either with medium or with DNP_6OVA. UCA (trans or cis isomer) was added to the cultures at day 0 at the final concentration indicated.

[b] In cpm $\times 10^3$; figures in parentheses represent SEM.

[c] After 3 days of culture, uptake of [^{3}H]Thymidine after an overnight pulse of 1 μCi/culture was determined. [^{3}H]Thymidine incorporation (in cpm) in absence of DCs was: experiment 1: T cells alone, 4.5 (0.6); T cells + 100 μg/ml DNP_6OVA, 9.5 (0.4). Experiment 2: T cells alone, 3.2 (0.1); T cells + DNP_6OVA, 4.9 (0.2).

cytes) from UV-irradiated mice have a decreased APC activity. The question of whether purified splenic DC from UV-irradiated mice have defective antigen presenting function had not previously been addressed directly. Purified DCs were prepared from the spleens of normal mice or from mice that had been given 1 h (27 kJ/m²) of UVB radiation from FS40 sunlamps 7 days earlier. This dose of UV is sufficient to cause 70%–75% systemic suppression of contact hypersensitivity [1]. As shown in Fig 1, antigen pulsed DCs from UV-irradiated mice had significantly lowered ability to stimulate T cell proliferation at all of the three DC concentrations tested. At two of the three DC concentrations tested, the syngeneic proliferative response to DCs from UV mice was also significantly lowered compared to that obtained with DC from untreated mice.

Antigen Presenting Function of Splenic Dendritic Cells after Administration of Cis-Urocanic Acid or Trans-Urocanic Acid Intravenously To establish if administration of cis-UCA could mimic the effect of UV irradiation on antigen-presenting cell function, cis-UCA or trans-UCA was administered intravenously and the splenic DCs were harvested 7 days later. Results of two proliferation experiments using these cells as APC are given in Figure 2*A*, 2*B*, and 2*C*. Administration of 100 μg or 200 μg/mouse of cis-UCA significantly decreased the ability of DCs to present antigen at all three concentrations of DCs investigated (Fig 2*A* and 2*B*). Administration of 50 μg/mouse of cis-UCA significantly decreased APC function only at two of three concentrations of DCs tested (Fig 2*C*). Administration of 50–200 μg/mouse of trans-UCA did not significantly decrease APC function at any concentration of DCs tested (Fig 2). In one experiment, (Fig 2*B*, 2*C*), the syngeneic proliferative response was also significantly lowered with the highest concentration of DCs tested (2×10^4) from cis-UCA-treated mice, compared to the response elicited by the same number of DC from normal mice.

In none of these studies was there any detectable toxicity of either isomer of UCA in vitro or in vivo. Mice given UCA show no symptoms of distress or loss of weight, confirming earlier studies that found neither isomer of UCA is toxic to mice [24]. Spleens from UCA treated mice are macroscopically normal, and histologic sections showed no evidence of necrosis or cell damage (data not shown).

The yield of DCs from UV-treated mice or from mice given cis-UCA was in a number of experiments 20%–25% lower than the yield of DCs from normal mice or from mice given trans-UCA, but this difference is of doubtful significance in view of the small number of DCs obtained per mouse ($1-5 \times 10^5$) and because the yields of DCs varied considerably between experiments.

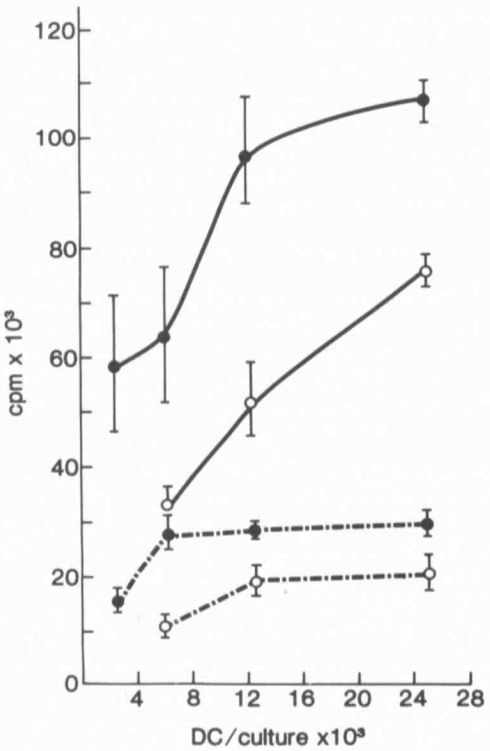

Figure 1. Ability of splenic DCs from normal or UV-irradiated mice to present antigen in a proliferation assay. Responder cells: 4×10^5 purified T cells from mice immunized with DNP_6OVA in CFA (Materials and Methods). Stimulator cells: DCs (prepared as described in Materials and Methods) from normal mice *(filled circles)* or from mice given 1 h (27 kJ/m²) of UV radiation *(open circles)* 7 days previously. DCs were pulsed either with medium *(dotted lines)* or with 100 μg/ml of DNP_6OVA *(solid lines)* prior to culture. [^{3}H]Thymidine incorporation (cpm ± SE) shown after 3 days of culture. Response to DCs pulsed with antigen from UV irradiated mice was significantly lower than response to corresponding DCs from normal mice at all three DC concentrations shown ($p < 0.025$). For 6×10^3 and 24×10^3 DCs pulsed with medium from UV-treated mice, the response was significantly lower ($p < 0.025$) than response to corresponding numbers of DCs pulsed with medium for normal mice. Proliferative response of T cells in absence of DCs: T cells alone, 3.5 (0.2); T cells + 100 μg/ml DNP_6OVA, 9.1 (1.1).

Characteristics of Dendritic Cells from Ultraviolet Irradiated and from Urocanic Acid-Treated Mice

Fc Receptors Dendritic cells used in the experiments described above were prepared as detailed in Materials and Methods by BSA flotation and by adherence. It was possible that differential contamination with FcR+, Ia+ cells in the DC preparations from each experimental group was responsible for the observed differences in APC activity. Dendritic cells from untreated mice, from mice given 1 h of UV irradiation or 100 μg of cis- or trans-UCA i.v. 7 days previously were rosetted with SRBC opsonized with anti-SRBC antibody. The percentages of rosetting cells (Fc receptor positive) in the DC preparations were: untreated, 11%; UV, 8%; trans-UCA, 8%; cis-UCA, 10%. These figures indicate no significant differences between experimental groups. Further, (data not shown) APC functional studies of DC preparations depleted of FcR+ cells by rosetting and by reflotation on BSA, showed that Fc receptor-negative DCs from UV-irradiated or cis-UCA-treated mice retained a decreased ability to present antigen in a proliferation assay relative to DC from untreated mice or from mice given trans-UCA.

Ia Expression Because expression of surface Ia antigen on APC has a pivotal role in antigen presentation, and because modulation of Ia

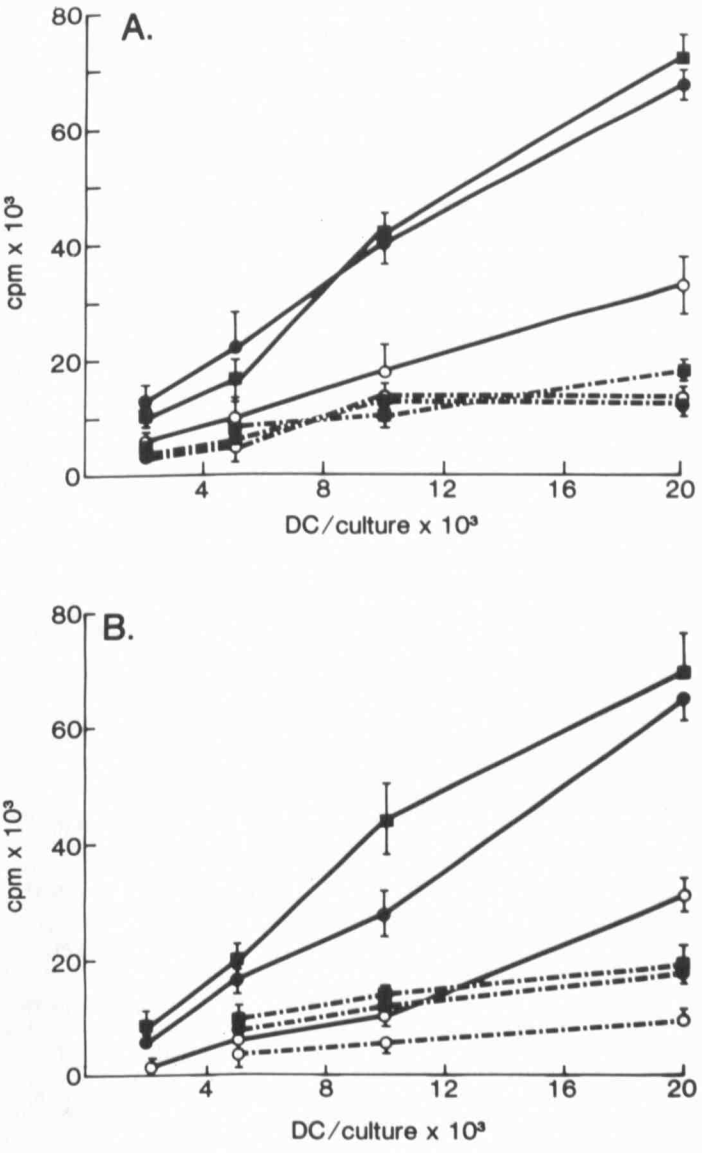

Figure 2. Effect of prior administration of cis- or trans-UCA in vivo on the ability of DCs to present antigen in a proliferation assay. Conditions as for Fig 1 except that 200 μg/mouse *(A)*, 100 μg/mouse *(B)*, or 50 μg/mouse *(C)* of cis-UCA or trans-UCA was administered i.v. 7 days prior to harvesting DCs. [³H]Thymidine uptake (cpm ± SE) after 3 days of culture using DCs from normal mice *(filled circles)*, from mice given cis-UCA *(open circles)*, or trans-UCA *(filled squares)*, pulsed with medium *(dotted lines)* or with DNP_6OVA *(solid lines)* prior to culture.

For *A*, response to DCs pulsed with antigen from cis-UCA-treated mice was significantly lower than response than to DCs pulsed with antigen from normal mice ($p < 0.01$ for 10 and 20×10^3 DCs; $p < 0.05$ for 5×10^3 DCs; $p < 0.025$ for 2.5×10^3 DCs). Proliferative response of T cells in absence of DC: T cells alone, 3.2 (0.1); T cells + DNP_6OVA, 4.9 (0.2). For *B*, response to DCs pulsed with antigen from cis-UCA-treated mice was significantly lower than response to corresponding DC from normal mice at all three DC concentrations ($p < 0.01$). Syngeneic proliferative response (to DCs pulsed with medium) was significantly lower for DCs from cis-UCA-treated mice than for DCs from normal mice at highest DC concentration only ($p < 0.01$).

For *C*, response to antigen pulsed DCs from cis-UCA mice was significantly lower than that of corresponding DCs from normal mice at 20×10^3 DCs and 5×10^3 DCs ($p < 0.01$). Syngeneic proliferative response as for *B*. For *B* and *C*, proliferation of T cells in absence of DC: T cells alone, 3.2 (0.1); T cells + DNP_6OVA, 4.9 (0.2).

expression on at least certain subsets of APC is critical in regulation of the immune response, we investigated the expression of Ia antigen on DC preparations from different treatment groups. We used FACS analysis of DCs stained in a two-stage procedure with a monoclonal antibody to IA^d [23] and a fluorescent-labeled second antibody. As shown in Fig 3*A*, intensity of IA expression on DC from normal and from UV mice and the percentage of IA+ cells in the preparations are similar. In Fig 3*B*, expression of IA^d on DCs from normal, cis-UCA- or trans-UCA-treated mice is the same, and the percentage of IA+ cells in the preparations is 87%–96%. These observations indicate that the APC alteration mediated by cis-UCA is not associated with an alteration to constitutive Ia expression on DC.

Production of Suppressive Factors We next investigated if DCs from UV- or from cis-UCA-treated mice could alter the APC ability of DCs from normal mice. As shown in Table II, mixture of DCs from either UV-treated mice or cis-UCA-treated mice together with DCs from normal mice did not depress the proliferation response. Further, addition of indomethacin (5 μg/ml) to cultures with DCs from trans-UCA- or cis-UCA-treated mice did not significantly alter the proliferation response, indicating prostaglandin secretion was not responsible for the decrease in APC function of DCs from cis-UCA-treated mice (Table III).

Time Course of Alteration of Dendritic Cell Function after Ultraviolet Irradiation or After Cis-Urocanic Acid Administration. One of the characteristics of UV-induced suppression is a time delay of 1–3 days in its generation and a persistence of up to 15 days after a single dose of UV irradiation [14]. We showed previously [14] that the antigen-presenting cell defect in UV-irradiated mice—determined by the ability of TNP-conjugated splenic adherent cells to prime mice for a DTH response—also required 3 days after UV irradiation to be generated. We therefore investigated if the effect of UV radiation or cis-UCA on APC ability of DCs also showed a time delay. As shown in Table IV, DCs taken 3 days after either UV irradiation or cis-UCA administration had normal APC ability, assessed by their ability to stimulate an antigen-specific proliferative response. In contrast, within the same experiments, DCs taken 7 days after UV irradiation or 8 days after cis-UCA administration, respectively, had decreased APC function, indicating a time delay of 3–7 days after UV irradiation or cis-UCA treatment in generation of the APC defect.

DISCUSSION

The essential finding of this study is that intravenous administration of the UVB-induced isomer of urocanic acid, cis-UCA, like in vivo UVB irradiation, depresses the antigen-presenting function of

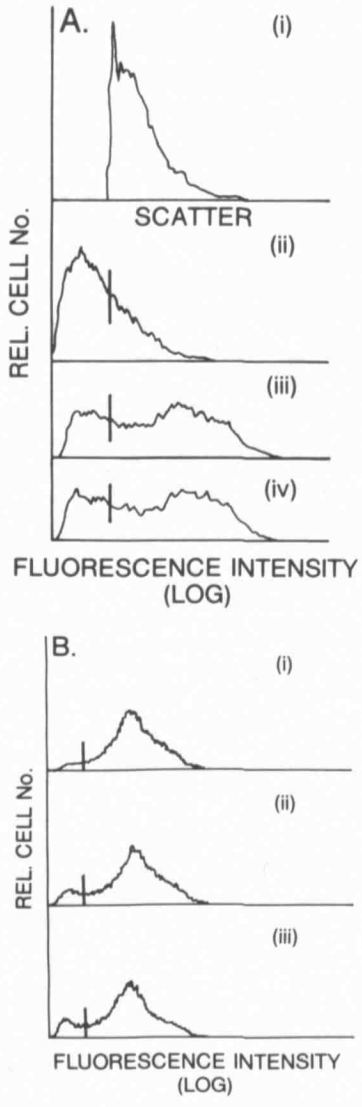

Figure 3. Density of surface IA^d antigen detected by FACS analysis of DCs from normal, UV- or cis- or trans-UCA-treated mice. *A*: *(i)*. Scatter histogram for DCs from normal or UV-treated mice (27 kJ/m² 7 days previously). *(ii)*. Fluorescence histogram for DCs from normal or UV-treated mice reacted with FITC goat anti-mouse antibody. *(iii)*. Fluorescence histogram for DCs from normal mice reacted with anti-IA^d hybridoma supernatant and FITC goat anti-mouse antibody. *(iv)*. Fluorescence histogram for DC from UV mice treated as in *(iii)*. The % of IA+ cells calculated from these plots are: Normal, 68%; UV, 65%.

B: *(i)*. Fluorescence histogram of DCs from normal mice treated as in A *(iii)*. *(ii)*. As above, but for DCs from mice given 200 μg/mouse of cis-UCA i.v. 7 days previously. *(iii)*. As above, but for DCs from mice given 200 μg/mouse of trans-UCA i.v. 7 days previously. The DCs were the same preparations as used for the experiment described in Figure 2*A*. Less than 10% of cells were weakly positive with FITC goat anti mouse antibody alone. The percentages of IA positive cells calculated from these plots are: normal, 96%; cis-UCA, 89%, trans-UCA, 87%.

Table II. Mixing of Dendritic Cells from Ultraviolet Radiation-Mice or from cis-Urocanic Acid-Treated Mice with Dendritic Cells from Normal Mice Does Not Depress Antigen Presentation

	DCs Added to Culture[a]		
Experiment	Treatment	DCs/culture ($\times 10^4$)	[³H]Thymidine Incorporation[b,c]
1	Normal, pulsed with medium	1.6 0.8	7.7 (0.6) 6.3 (0.6)
	Normal, pulsed with DNP_6OVA	1.6 0.8	30.6 (1.5) 21.4 (1.5)
	UV, pulsed with medium	1.6 0.8	10.1 (0.5) 5.9 (0.4)
	UV, pulsed with DNP_6OVA	1.6 0.8	23.0 (0.6)[d] 14.6 (0.8)[d]
	Normal, pulsed with DNP_6OVA + Normal, pulsed with medium	1.6 + 0.8	32.0 (1.4)
	Normal, pulsed with DNP_6OVA + UV, pulsed with medium	1.6 + 0.8	34.0 (3.8)
2	Normal, pulsed with medium	1.0	14.8 (1.9)
	Normal, pulsed with DNP_6OVA	1.0	70.8 (3.5)
	Trans-UCA, pulsed with medium	1.0	18.8 (0.8)
	Trans-UCA, pulsed with DNP_6OVA	1.0	75.2 (6.4)
	Cis-UCA, pulsed with medium	1.0	15.3 (1.7)
	Cis-UCA, pulsed with DNP_6OVA	1.0	53.5 (3.3)[d]
	Normal, pulsed with DNP_6OVA + Trans, pulsed with medium	1.0 + 0.5	84.6 (0.8)
	Normal, pulsed with DNP_6OVA + Cis, pulsed with medium	1.0 + 0.5	85.9 (7.3)

[a] Conditions as for Table I: 4×10^5 purified T cells from mice immunized with DNP_6OVA cultured with DCs as indicated from mice given 1 h of UV radiation (experiment 1), or 100 μg/mouse of trans-UCA or cis-UCA i.v. (experiment 2), 7 days previously.

[b] In cpm $\times 10^3$; figures in parentheses represent SEM.

[c] As for Table I. [³H]thymidine incorporation (in cpm) in absence of DCs: experiment 1: T cells alone, 4.3 (0.2); T cells + DNP_6OVA, 7.4 (0.7). Experiment 2: T cells alone, 4.9 (1.5); T cells + DNP_6OVA, 21.0 (3.0).

[d] Significantly different ($p < 0.05$) from response obtained with the same number of corresponding DCs from normal mice.

splenic dendritic cells. These studies were done with purified populations of splenic DCs, in contrast to earlier studies demonstrating an APC defect in spleen cells from UV-treated mice that had used heterogenous cell populations as APC. Administration of cis-UCA in vivo has thus been shown to mimic one of the systemic effects of UV radiation on the immune system. These investigations support our hypothesis [9,34,35] that an interaction between UV radiation and UCA in the skin initiates systemic immune suppression by way of an APC alteration. The doses of cis-UCA used in the current study (50–200 μg or 0.36–1.45 μmoles/mouse) are within the biologic range: that is, they approximate the amounts of cis-UCA that are generated in mouse skin in vivo by immunosuppressive doses of UVB radiation.

The finding that purified splenic dendritic cells from UV-irradiated mice have a decreased ability to present antigen in vitro is a new observation. Previous studies (including our own) found that splenic adherent cells (a mixture of macrophages, dendritic cells, and lymphocytes) from UV-irradiated mice have a decreased ability to present antigen both in vivo [13,14] and in vitro [25]. Because purified populations of APC were not used, it was difficult to establish from these experiments the mechanism by which the APC defect is generated.

Letvin et al [25] found that in vivo UV irradiation is associated

Table III. Addition of Indomethacin to Cultures Does Not Alter the Antigen-Presenting Activity of Dendritic Cells from Urocanic-Acid-Treated Mice

DCs Added to Culture[a]		
Treatment	DCs/culture ($\times 10^4$)	[^{3}H]Thymidine Incorporation[b,c]
Normal, pulsed with medium	1.0	14.8 (1.9)
Normal, pulsed with DNP_6OVA	1.0	70.8 (3.5)
Trans-UCA, pulsed with medium	1.0	18.8 (0.8)
Trans-UCA, pulsed with DNP_6OVA	1.0	75.2 (6.4)
as above + 5 μg/ml indomethacin	1.0	85.3 (5.1)
Cis-UCA, pulsed with medium	1.0	15.3 (1.7)
Cis-UCA, pulsed with DNP_6OVA	1.0	53.5 (3.3)[d]
Cis-UCA, pulsed with DNP_6OVA + 5 μg/ml indomethacin	1.0	62.6 (6.1)[e]

[a] Conditions as for Table 1: 4×10^5 purified T cells from mice immunized with DNP_6OVA cultured with DCs, as indicated, from mice given 100 μg/mouse of trans-UCA or cis-UCA i.v. 7 days previously.

[b] In cpm $\times 10^3$; figures in parentheses represent SEM.

[c] As for Table I: [^{3}H]thymidine incorporation (in cpm) in absence of DCs: T cells alone, 4.9 (1.5); T cells + DNP_6OVA, 21.0 (3.0).

[d] Significantly different ($p < 0.05$) from response obtained with the same number of corresponding DCs from normal mice.

[e] Significantly different ($p < 0.05$) from response obtained with corresponding DCs from trans-UCA-treated mice, cultured with indomethacin.

with a net loss of adherent Ia-bearing cells from the spleen, indicating that the observed APC defect could be due to a redistribution of Ia-positive cells from the spleen, or to alterations in Ia expression on at least a subset of splenic cells. The current study has shown, however, that alterations to Ia expression or redistribution of one subset of APC are not necessary correlates of the APC defect found in UVB-irradiated animals. Our findings presented in this paper indicate that, at least for splenic dendritic cells (which are very potent antigen-presenting cells), a per-cell decrease in APC ability occurs after UVB irradiation or cis-UCA treatment. Dendritic cells from UV-irradiated- or cis-UCA-treated mice, when pulsed with antigen and cultured with immune T cells, stimulated significantly lower proliferative responses than did similar numbers of corresponding DCs from normal mice or from mice given trans-UCA. In spite of these differences in activity, purified DCs from normal, UV-irradiated, cis-UCA- or trans-UCA-treated animals were similar in cell surface markers (>65% Ia positive, 8%–11% Fc receptor-positive), and in intensity of Ia expression. We were also unable to establish significant decreases in the yield of DCs from UV-irradiated or cis-UCA-treated mice.

Dendritic cells may not be the only APC functionally altered by in vivo UV irradiation. Preliminary results (Noonan et al., unpublished) indicate that plastic-adherent resident peritoneal cells from UV irradiated mice are less effective at presenting antigen than are comparable cells from normal mice, and that this loss in APC function can also be brought about by in vivo administration of cis- but not of trans-UCA.

The finding of an alteration in the ability of DCs from UV-treated mice to present antigen strengthens the hypothesis for a central role of an APC defect in UV-induced suppression. We have not in this investigation been able to find any differences in the functional alteration to DCs caused by UV and by cis-UCA. Both are independent of alterations to constitutive Ia expression, both show a similar time course, and there is no evidence in either case for production of suppressive substances by the DCs. Little is known about transport of cis-UCA systemically from its site of generation in the skin, and further investigation of this point is necessary to fully understand the mechanism of in vivo UV-induced suppression.

What might be the mechanism by which cis-UCA alters APC function? Constitutive Ia expression is unaltered in DCs from UV- or cis-UCA-treated mice. The action of cis-UCA does not, therefore, mimic the effect of prostaglandins, glucocortoids, or α-fetoprotein, which down regulate APC function by decreasing Ia expression [31,32]. It is, however, possible that the enhanced Ia expression that occurs on activation of APC during antigen presentation is blocked in APC from UV- or cis-UCA-treated mice. We have found no evidence for suppressive effects of DCs from UV or cis-UCA-treated mice when mixed with DCs from normal mice, in accord with the findings of Greene et al [13] who used splenic adherent cells from UV-irradiated mice. Therefore, other mechanistic possibilities that remain to be investigated include alterations to antigen uptake and/or processing, alterations to the association between T cells and DCs, and alterations to production of accessory factors (eg, interleukin-1). As indicated in Results, and borne out by the data in Table I (which indicate that UCA added in vitro did not alter a proliferation response), we have found no toxic effect of cis-UCA in vivo or in vitro, confirming earlier observations [24].

Other factors mediating UV-induced suppression of CHS have been described. Swartz (26) found that mouse serum taken 2–6 h after UV irradiation could transfer suppression of CHS with the generation of suppressor cells. Schwartz et al [27] described a factor produced by the UV irradiation of epidermal cells that could suppress CHS and that may be the anti-IL-1 factor also described by this group. It appears that none of the factors described in these two papers can be free cis-UCA because the molecular weights are too

Table IV. Time Course of Depression of Antigen-Presenting Cell Function after Ultraviolet Irradiation or after Cis-Urocanic Acid Treatment

DCs Added to Culture[a]		[^{3}H]Thymidine Incorporation[b,c]	
Treatment of Donors	Time between Treatment and DC Harvesting (days)	DC Pulsed with Medium	DC Pulsed with DNP_6OVA
None	—	25.5 (2.9)	80.8 (4.8)
UV	3	28.1 (2.1)	89.5 (1.9)
UV	7	15.5 (1.9)	35.7 (2.5)[d]
cis-UCA	7	19.5 (1.9)	42.0 (2.3)[d]
None	—	24.1 (2.9)	112.4 (4.2)
cis-UCA	3	13.0 (0.9)	99.0 (16.0)
cis-UCA	8	30.3 (3.9)	66.8 (7.4)[d]
trans-UCA	8	24.0 (3.5)	90.0 (5.6)

[a] As for Table I: 4×10^5 purified T cells from mice immune to DNP_6OVA cultured with 1×10^4 DC (experiment 1, upper data) or 2×10^4 DCs (experiment 2, lower data), from mice given 1 h of UV irradiation or 100 μg/mouse of UCA.

[b] In cpm $\times 10^3$; figures in parentheses represent SEM.

[c] As for Table I: [^{3}H]thymidine incorporation (in cpm) in the absence of T cells: experiment 1: T cells alone, 4.1 (0.8); T cells + DNP_6OVA, 12.5 (2.4). Experiment 2: T cells alone, 4.5 (0.8); T cells + DNP_6OVA: 9.4 (1.1)

[d] Significantly different ($p < 0.01$) from value obtained with corresponding DC from untreated mice.

high. It will be of interest, however, to establish if the generation of any of these factors by UV irradiation is related to the UV-induced formation of cis-UCA in the skin. In another study, Chung et al [28] found that administration of very high doses of indomethacin in vivo (in slow-release pellets) prevented the generation of UV-induced suppression, suggesting that prostaglandins played an essential role in the generation of suppression. These results would appear to be at variance with our data (Table III), which indicated that inclusion of indomethacin in the cultures did not alter the antigen presenting cell defect. Our experiments do not, however, exclude the possibility that prostaglandins may act at a stage in UV-induced suppression in vivo not detected with in vitro assay systems (eg, in the generation or action of suppressor cells). Only further experimentation will resolve this point.

Our own studies have investigated the systemic suppressive effects of UV radiation. Studies from other laboratories, originally those of Toews et al [29] have shown that UV irradiation also has a locally suppressive effect: that is, suppression of CHS occurs when the contact sensitizer is applied directly to a UV-irradiated site instead of to an unirradiated site as for systemic suppression. The UV radiation doses required and the kinetics of local suppression differ somewhat from those for systemic suppression, and it is not clear whether the mechanisms of suppression are the same for both systems. The data of Ross et al [30] (discussed below), however, indicate that cis-UCA may also play a role in the generation of local suppression of DTH.

A time delay of 3–7 days was observed in the generation of the APC defect in DCs by UV radiation or cis-UCA. Preliminary results (not shown) indicated that DCs taken from mice 15 days after UV irradiation or after cis-UCA administration had normal antigen-presenting ability. This time delay is, however, puzzling because in 7 days, according to Steinman et al (33), splenic DCs are almost completely replenished. It is therefore possible that cis-UCA may alter the recruitment and/or maturation of DCs, a point that warrants further study. In our previous studies of the time course of systemic suppression of CHS by UV radiation, we noted that CHS was suppressed when antigen was applied 3 days after UV radiation [14]. In the generation of the antigen-presenting cell defect described in this paper, splenic DCs showed a depressed APC ability only 7 days after UV treatment. Although unclear, it is possible that differences in methodology account for this discrepancy (ie, CHS is measured over 4–5 days in vivo, whereas APC function in vitro occurs in less than 24 h). Another possibility is that CHS priming occurs primarily in the draining lymph nodes and that the kinetics of alteration of APC function in the lymph nodes by UV radiation or cis-UCA differs from the effect on splenic DCs. Complex interactions are likely to be involved in generation of suppression, however, because hapten conjugated APC taken from UV-treated mice 3 days after irradiation can prime normal mice for DTH, but cannot prime UV-treated mice. Further study, particularly of the APC function of DCs from the draining lymph nodes may resolve some of these questions.

At the molecular level, it is of interest to note that both cis- and trans-UCA are analogues of histidine and of histamine, by virtue of an imidazole ring. The structural differences between trans- and cis-UCA reside in the spatial distribution of the carboxyl group around the double bond in the side chain of the molecule. It should be pointed out that, although trans-UCA is metabolized by the enzyme urocanase, this enzyme does not appear to metabolise cis-UCA (Hug, personal communication). Thus, the observed differential in activity between trans- and cis-UCA may be due to the fact that trans-UCA is metabolized and cis-UCA is not, or due to a specific interaction of cis-UCA with some receptor molecule that does not react with trans-UCA.

In a previous preliminary study [35], we found suppression of a contact hypersensitivity response (up to 50% suppression) by intraperitoneal administration of cis-UCA, with no effect of trans-UCA. Recently, Ross et al [30] have found that administration of UV-irradiated UCA (a mixture of trans and cis isomers) via the skin substantially suppresses the delayed type hypersensitivity response to herpes virus type I with the generation of suppressor T cells, paralleling their previous findings of UV-induced suppression of this response [3]. Taken together, this study and our own investigations strongly support our original hypothesis that UVB-induced suppression is initiated by UVB-induced isomerization of trans-UCA to cis-UCA in the skin. The current study further provides supportive evidence that this suppression occurs via a defect in the function of antigen presenting cells.

In conclusion, evidence has been presented supporting our hypothesis that UVB-induced immune suppression in vivo is initiated by the photoisomerization of UCA in the skin. The data suggest that the UV-irradiated form of UCA (cis-UCA) initiates immune suppression by causing an antigen presenting cell defect in vivo. This is the first evidence that purified cis-UCA can interact with the immune system, indicating that this compound has a potential role as an immunomodulating agent. Further studies with cis-UCA may lead to an understanding of mechanisms by which suppressor cells are formed and regulated, and may lead to a better understanding of UV radiation-related diseases.

The excellent technical assistance of Ms Helen Zowtyj and Ms Rota Koy is gratefully acknowledged. Thanks are also due to Drs. Keryn Williams, Heddy Zola, Ira Green, and Ethan Shevach for helpful discussions. Particular thanks are due to Drs. J. J. Finlay-Jones and P. J. McDonald, whose support and encouragement made these studies possible. We also wish to thank Dr. M. E. Elgart for his encouragement and support.

REFERENCES

1. Noonan FP, De Fabo EC, Kripke ML: Suppression of contact hypersensitivity by UV radiation and its relationship to UV-induced suppression of tumor immunity. Photochem Photobiol 34:683–690, 1981
2. Lynch DH, Gurish MF, Daynes RA: The effects of high-dose UV exposure on murine Langerhans cell function at exposed and unexposed sites as assessed using in vivo and in vitro assays. J Invest Dermatol 81:336–341, 1983
3. Howie SEM, Norval M, Maingay J, Ross JA: Two phenotypically distinct T cells (Ly 1+2− and Ly 1−2+) are involved in ultraviolet B light induced suppression of the efferent DTH response to HSV-1 in vivo. Immunology 58:653–658, 1986
4. Giannini MSH: Suppression of pathogenesis in cutaneous Leishmaniasis by UV irradiation. Infect Immun 51:838–843, 1986
5. Fisher MS, Kripke ML: Systemic alteration induced in mice by ultraviolet light irradiation and its relationship to ultraviolet carcinogenesis. Proc Natl Acad Sci USA 74:1688–1692, 1977
6. Spellman CW, Daynes RL: Modification of immunologic potential by ultraviolet radiation. II. Generation of suppressor cells in short term UV irradiated mice. Transplantation 24:120–126, 1977
7. Ansel JC, Mountz J, Steinberg AD, De Fabo EC, Green I: Effects of UV radiation on autoimmune strains of mice: Increased mortality and accelerated autoimmunity in BXSB male mice. J Invest Dermatol 85:181–186, 1985
8. Hauser SL, Weiner HL, Che M, Shapiro ME, Gilles F, Letvin NL: Prevention of experimental allergic encephalomyelitis (EAE) in the SJL/J mouse by whole body ultraviolet irradiation. J Immunol 132:1276–1281, 1984
9. De Fabo EC, Noonan FP: Mechanism of immune suppression by UV irradiation in vivo. I. Evidence for the existence of a unique photoreceptor in skin and its role in photoimmunology. J Exp Med 158:84–98, 1983
10. Scott IR: Factors controlling the expressed activity of histidine-ammonia lyase in the epidermis, and the resulting accumulation of urocanic acid. Biochem J 194:829–838, 1981
11. Morrison H, Avnir D, Bernasconi C, Pandey G: Z/E photoisomerization of urocanic acid. Photochem Photobiol 32:711–714, 1980
12. Baden HP, Pathak MA: The metabolism and function of urocanic acid in skin. J Invest Dermatol 48:11–17, 1967
13. Greene MI, Sy MS, Kripke ML, Benacerraf B: Impairment of antigen presenting cell function by ultraviolet radiation. Proc Natl Acad Sci USA 76:6592–6595, 1979

14. Noonan FP, Kripke ML, Pedersen GM, Greene MI: Suppression of contact hypersensitivity in mice by ultraviolet irradiation is associated with defective antigen presentation. Immunology 43:527–533, 1981
15. Noonan FP, De Fabo EC, Kripke ML: Suppression of contact hypersensitivity by ultraviolet irradiation: an experimental model. Springer Seminars Immunopathol 4:293–304, 1981
16. Morrison H, Avnir D, Zarrella T: Analysis of Z and E isomers of urocanic acid by high performance liquid chromatography. J Chromatog 183:83–86, 1980
17. Caron JC, Martin B, Schroot B: High-performance liquid chromatographic determination of urocanic acid isomers in biologic samples. J Chromatog 230:125–130, 1982
18. Steinman RN, Cohn Z: Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties in vitro. J Exp Med 139:380–397, 1974
19. Eisen HN: In: Williams CA, Chase MW (eds): Methods in Immunology and Immunochemistry. vol 1. New York Academic Press, p 130
20. Julius MH, Simpson E, Herzenberg LA: A rapid method for isolation of functional thymus derived murine lymphocytes. Eur J Immunol 3:645–649, 1973
21. Ly IA, Mishell RI: Separation of mouse spleen cells by passage through columns of Sephadex G-10. J Immunol Methods 5:239–247, 1974
22. Stingl G, Gazze-Stingl LA, Aberer W, Wolff K. Antigen presentation by murine epidermal Langerhans cells and its alteration by ultraviolet B light. J Immunol 127:1707–1713, 1981
23. Kappler JW, Skidmore B, White J, Marrack P: Antigen-inducible H-2 restricted, interleukin 2 producing T cell hybridoma. J Exp Med 153:1198–1124, 1981
24. Kurogochi Y, Fukui T, Nakagawa T, Yamamoto I: A note on cis-urocanic acid. Jap J Pharmacol 6:147–150, 1957
25. Letvin NL, Nepom JT, Greene MI, Benacerraf B, Germain RN: Loss of Ia-bearing splenic adherent cells after whole body ultraviolet irradiation. J Immunol 125:2550–2554, 1980
26. Swartz RP: Role of UVB-induced serum factor(s) in suppression of contact hypersensitivity in mice. J Invest Dermatol 83:305–307, 1984
27. Schwartz T, Urbanska A, Gschnait F, Luger TA: Inhibition of the induction of contact hypersensitivity by a UV-mediated epidermal cytokine. J Invest Dermatol 87:289–291, 1986
28. Chung H-T, Burnham DK, Robertson B, Roberts LK, Daynes RA: Involvement of prostaglandins in the immune alterations caused by the exposure of mice to ultraviolet radiation. J Immunol 137:2478–2484, 1986
29. Toews GB, Bergstresser PR, Streilein JW: Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. J Immunol 124:445–449, 1980
30. Ross J, Howie SEM, Norval M, Maingay J, Simpson T: UV irradiation of urocanic acid suppresses the delayed type hypersensitivity response to *H. simplex* virus in mice. J Invest Dermatol 87:630–633, 1986
31. Snyder DS, Beller DI, Unanue ER: Prostaglandins modulate macrophage Ia expression. Nature 299:163–165, 1982
32. Lu CY, Changelain PS, Unanue ER: Alpha-fetoprotein inhibits macrophage expression of Ia antigen. J Immunol 132:1722–1727, 1984
33. Steinman RM, Lustig DS, Cohn ZA: Identification of a novel cell type in peripheral lymphoid organs of mice. III. Functional properties in vivo. J Exp Med 139:1431–1435, 1974
34. Noonan FP, De Fabo EC: Immune suppression by ultraviolet radiation and its role in ultraviolet radiation induced carcinogenesis in mice. Aust J Dermatol 26:4–8, 1985
35. Hug DH, Morrison H, Tessman I, De Fabo EC, Noonan FP: Photobiology of urocanic acid. In: Longworth JW, Jagger J, Shropshire W Jr (eds): Photobiology 1984, Praeger, New York, p 141–146, 1985